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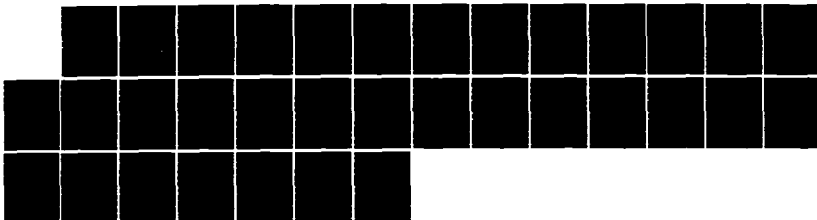
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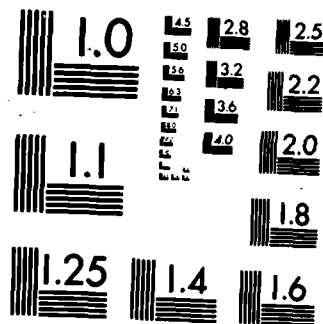
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FINAL SCIENTIFIC REPORT ON GRANT AFOSR-82-0100

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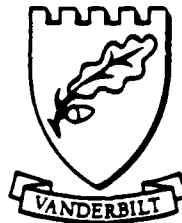
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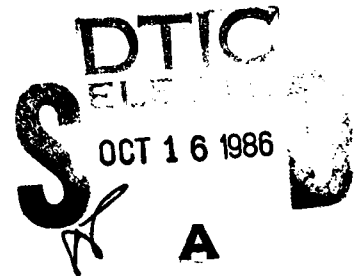


Submitted to the
Air Force Office of Scientific Research

by

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FINAL TECHNICAL REPORT FOR GRANT AFOSR-82-0100

Abstract

Research under this grant has developed a theoretical framework for understanding the structures and properties of a broad class of enzyme inhibitors and related compounds. In particular it has focused on the use of *ab initio* computations to study pertinent structural parameters, the number and relative stabilities of molecular conformations, and the thermodynamics of reactions related to the function of esterase enzymes. Systems studied include: (1) actual inhibitors as well as smaller related compounds that serve as prototypes, (2) organic species related to the hydrolysis process both by enzymes and in aqueous solution, and (3) a key reactivating compound. The net result of this project has been not only a considerable volume of quantitative data concerning molecular and ionic structures and energetics that bear directly on all aspects of esterase enzymes, their inhibitors and inhibitor antidotes, but also a set of general principles describing the results, which may be generalized to a number of chemically related systems.

I. Introduction

During the past few years the range of practical chemical problems that may be usefully addressed by nonexperimental computational techniques has grown at a rapidly increasing rate. Since such techniques require in principle no experimental input data, while often yielding results that are of immediate practical application, they are particularly well suited for studying systems which are relatively impractical (or unsafe) to treat experimentally. Because we see the role of nonexperimental computations becoming increasingly vital to nearly all branches of research relating to the properties of matter on a molecular level - including several areas of practical interest to the Air Force

- our group has for the past several years contributed to the development, application and advocacy of such research.

The present project, carried out under grant AFOSR-82-100, presented a challenge to the state of the art in nonempirical molecular computations, with respect to both the size of the species involved and the levels of accuracy required. The project consisted of the application of quantum-theoretical (*ab initio*) methods to a number of studies related to enzyme inhibitors and chemically related compounds. The objective was to characterize a broad and practically important class of inhibitors: the serine proteases and esterases, which hydrolyze peptide and ester linkages respectively. A particularly significant example of an esterase to which our findings may be applied is acetylcholinesterase, which hydrolyzes the acetylcholine neurotransmitter. Inhibitors of human acetylcholinesterase are virulent chemical warfare agents, often termed nerve agents.

The acetylcholinesterase enzyme binds strongly to either its natural substrate, acetylcholine or its hydrolysis intermediates, or an inhibitor molecule in a way that depends critically on the shape and size of the species being bound¹. In other words the molecular structure of the substrate or inhibitor must precisely complement that of the enzyme's active site in order for effective binding to take place. The same principle applies the enzyme-reactivating antidotes or prophylactic compounds, which must rely on the structure of the active site in order to achieve a high local concentration and hence to be effective.

In this research project we addressed the initial problem of determining the structural characteristics that lead to efficient enzyme binding by natural substrates, inhibitors and antidotes. This was done in most cases either by studying small prototype systems in considerable detail, or by examining actual compounds of practical importance. Specific goals were to develop, refine and test computational procedures, compute sets of quantitative data concerning structural parameters (bond lengths and angles, including torsional angles that determine conformations) and development more qualitative principles that may be applied to broad range of compounds that are not closely related to those chosen for study. In addition we carried out some systematic

studies concerning the energetics of reactions related to ester hydrolysis and binding to an esterase enzyme. In the next section of this report we summarize our conclusions concerning computational techniques that we developed or tested in carrying out this study. In the following section we review our findings categorized by type of compounds: (1) phosphorus acids and esters, including actual and prototype organophosphorus inhibitors, (2) prototypes of carboxylic ester hydrolysis intermediates, (3) reactivating inhibitor antidotes, and (4) additional studies whose bearing on this work involves the theory or technique involved, rather than the specific compounds studied. In each section we list some of the general conclusions to be drawn from our computed results. The last sections lists all publications resulting from this work.

The most significant set of findings resulting from this project may be summarized as follows. At its inception the detailed molecular structures, particularly conformations, of *none* of the three types of relevant species (substrates, inhibitors and antidotes) were known either theoretically or (with one or two exceptions) experimentally. At its conclusion the molecular structures of representative cases of *all three* types of species had been determined with sufficient precision and consistency to permit generalization to several other related species of practical importance.

II. Pertinent Computational Techniques

The first step in computing the properties needed to characterize any molecular system is to obtain the molecular energy and wavefunction at the self-consistent-field (SCF) level. In the past our group has developed and advocated orbital-partitioning techniques, such as those based on pseudopotentials, to decrease the computational effort required in this step by considering explicitly only the valence part of the electron distribution. However at an early stage of the research project it became clear that recent advances in programming to implement the older all-electron methods had more than offset the additional computer time they require due to their retaining nonvalence (core) electrons, at least for compounds containing elements no heavier than phosphorus. In particular the use of analytically computed gradients of the

energy with respect to molecular deformations has proven to be an extremely powerful way to obtain energy-optimized molecular structures. In addition the programs that have become available in recent years contain very efficient procedures (such as Møller-Plesset perturbation theory²) for estimating the electron-correlation corrections to the SCF approximation. The specific programs employed for most of our work were GAUSSIAN 80, GAUSSIAN 82, and our specially-adapted version of HONDO.

The molecules we studied, particularly the compounds of phosphorus, posed a considerable challenge to these molecular-structure optimization procedures. In some cases more than one hundred optimization cycles were required. In terms of the amount of computer time required, these are among the most ambitious such computations attempted by any research group to date. The computational effort stems largely from the lack of efficient and widely-applicable algorithms for deriving structures from the energies and gradients. The default GAUSSIAN 80 and 82 algorithm (the "Bernie" algorithm³) was found during the final stages of the project to be significantly less efficient for these molecules than an alternative procedure, the Murtagh-Sargent algorithm⁴. It is remarkable, considering the amount of effort that has been devoted to developing programs to compute the gradients, that the optimization procedures to exploit them are still fairly crude. We anticipate that the development of such algorithms will remain an active area of research for the next few years.

III. Computed Structures of Phosphorus Compounds

In this and the following three sections we briefly summarize our conclusions, categorized according to the compound or type of compound studied.

A. Methylphosphonyl Difluoride

It was clear at the outset of this research that there was extremely little accurate and unambiguous experimental data concerning the molecular structures of phosphoryl phosphorus compounds. However one such compound which has been thoroughly studied and for which there is reliable

experimental data⁵ is methylphosphonyl difluoride, $(\text{CH}_3)_2\text{F}_2\text{P}=\text{O}$. In Table I we list the structure computed at the SCF approximation in three types of basis sets.

Table I.
Structural Parameters of Methylphosphonyl Difluoride^a

Parameter	Calculated STO-3G	Calculated STO-3G*	Calculated 3-21G*	Experiment (microwave)
P=O	1.566	1.434	1.431	$1.442 \pm .009$
P-F	1.617	1.527	1.539	$1.544 \pm .011$
P-C	1.835	1.814	1.753	$1.795 \pm .019$
O=P-F	115.0	116.9	114.5	—
O=P-C	121.8	116.9	119.7	118.2 ± 1.5
P-C-H	110.1	110.1	111.4, 108.8	—
O=P-C-H	-2.1	-2.0	—	—

^a Bond lengths in Å, angles in degrees.

This table illustrates the order of accuracy which we believe is characteristic of all the other structures discussed below as well, i.e. bond lengths in error by 0.01 to 0.04 Å and angles by about 1°. Of particular interest is the role of the d polarization functions in the basis set employed to expand the molecular wavefunctions. The use of the d functions (denoted by the *), combined with a split-valence basis such as 3-21G or 4-31G was found to be the key to obtaining quantitative accuracy in all compounds of this type. In fact the errors in structures computed this way were found, in the majority of cases, to be of the same order as the experimental uncertainty (including uncertainties in interpreting experimental data, such as due to vibration).

A description of this work was reported in *Proceedings of the 1983 Scientific Conference on Chemical Defense Research*, (US Army Chemical Research and Development Center, Aberdeen Proving Ground, Maryland, 1984, 159)

B. Oxyacids

Our first systematic set of computations on a chemically and structurally related set of phosphoryl compounds were carried out on the simplest oxyacids, phosphinic ($\text{H}_2\text{P}=\text{O}(\text{OH})$), phosphonic ($\text{HP}=\text{O}(\text{OH})_2$), and phosphoric ($\text{P}=\text{O}(\text{OH})_3$) acids. This is clearly the logical starting point in any systematic study of phosphorus oxyacids and their esters. Our principal conclusions from this study are:

1. The structural parameters are given quite accurately in a relatively modest split-valence basis such as 3-21G* and 4-31G* (the * denoting d functions on the phosphorus only) when judged against those in a more elaborate 6-311G** basis. The latter, which includes two sets of d functions on phosphorus, one set of d's on oxygen and p functions on hydrogen, was developed by us for this comparison, since there are no relevant experimental gas-phase structure determinations.

2. The smaller STO-3G basis gives results of lesser but not unreasonable accuracy. However it tends to give incorrect numbers and ordering by energy of the stable molecular conformations.

3. The preferred equilibrium conformation for each species exhibits torsional angles that place the hydrogen of the OH group or groups in proximity with the phosphoryl oxygen. The distance between this hydrogen and oxygen is approximately constant between species, although the torsional angle varies between 0° in phosphinic acid and 42° in phosphoric acid.

4. In this and our subsequent related studies it was found that the torsional energy could be fit very accurately to a modified Fourier expansion of the form

$$V(\tau) = V_0 + V_1 \cos(\tau + \alpha_1) + V_2 \cos 2(\tau + \alpha_2) + V_3 \cos 3(\tau + \alpha_3)$$

where τ is the torsional angle and α is a phase angle, usually set to zero at an eclipsed orientation such as, in this case, when the acidic hydrogen is adjacent to the phosphoryl oxygen. The V_1 term measures the effect of electrical inhomogeneities such as dipole-monopole and dipole-dipole interactions. V_2 represents stereoelectronic effects such as hyperconjugation or "anomeric effects". V_3 measures steric repulsion. In phosphinic acid V_1 was found to be by far the largest term in each basis, indicating the predominance of electrostatic terms.

5. Computing the molecular dipole of each species as a function of torsional angle showed that in each case the structure with the lowest dipole moment exhibits the lowest energy. Based on this and the above observations we were able to prove that the structures of the phosphorus oxyacids are determined by simple electrostatic interactions. Although these acids,

particularly phosphoric acid, had previously been considered classic examples of the "anomeric" stereoelectronic effect,⁶ our alternative picture is now the generally accepted one.

6. Although x-ray diffraction studies⁷ had shown that these oxyacids had considerably differing structures in their crystals (due mainly to intermolecular hydrogen bonding) our results showed that the structures of the isolated molecules are all quite similar. In fact we found remarkable similarities in structural parameters, particularly bond lengths and angles associated with the phosphorus atom, in all the phosphoryl phosphorus compounds studied.

We have described this work in a recent paper published in *The Journal of the American Chemical Society* 107, 1965 (1985).

C. Methyl Esters of Oxyacids

We carried out a similar systematic study of the three simplest fully methylated esters of phosphorus oxyacids, methyl phosphinate ($\text{H}_2\text{P}=\text{O}(\text{OCH}_3)$), dimethyl phosphonate ($\text{HP}=\text{O}(\text{OCH}_3)_2$) and trimethyl phosphate ($\text{P}=\text{O}(\text{OCH}_3)_3$). The same methods were employed as in the preceding study, except that in this case no basis sets larger than 4-31G* was employed. The conclusions may be summarized as follows:

1. In contrast to the analogous acids, each ester has two or more stable conformations.

2. The most stable conformations of methyl phosphinate and dimethyl phosphonate are characterized by torsional angles that place the methyl adjacent to the phosphoryl oxygen. Trimethyl phosphate, however, has two adjacent and one opposed. This apparently the result of steric crowding as more bulky groups are attached to the phosphorus.

3. The Fourier expansion of the torsional potential of methyl phosphinate shows that the V_1 term is again the largest, indicating the role of dipolar electrostatic forces in determining the structure. The V_2 term, a measure of stereoelectronic effects, is less than 1/20 as large.

4. In methyl phosphinate and dimethyl phosphonate the structure with the smallest dipole moment is also the one with the least energy.

5. Trimethyl phosphate is the compound of greatest practical importance of this series, and the only one for which there is useful experimental data for comparison. Our structural parameters computed in the STO-3G* basis agree well with the only detailed experimental study⁸, which employed electron-diffraction spectroscopy. The primary exception is the set of torsional angles, which the latter reported as placing all the methyl groups in close proximity and opposed to the phosphoryl oxygen. Based on our experience with related compounds and the computed (and experimental) molecular dipole moments, we believe our lowest-energy computed structure, shown in Figure 1 below, is the correct one.

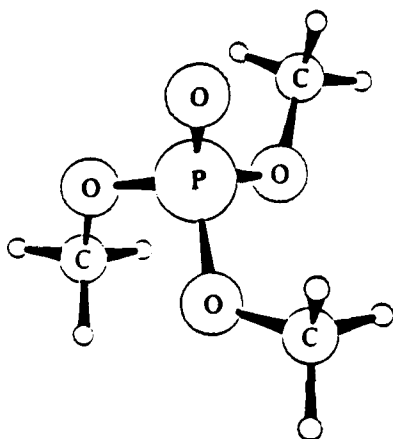


Figure 1.

6. Our computed dipole moment, averaged over our two computed conformations and weighted according to their relative energies, is in reasonable agreement with the experimental values in solution.

7. For the esterification reactions forming these three esters we compute gas-phase reaction enthalpies (including zero-point and thermal corrections) of -2.0, -2.7 and -9.2 kcal/mol respectively. The correction to the enthalpy due to interactions with water when these reactions are carried out in aqueous solutions is estimated⁹ to be +7 kcal/mol. So our computed enthalpies agree with the experimental values⁹ of +2 to +4 kcal/mol per ester linkage.

8. The most stable form of both dimethyl phosphonate and trimethyl phosphate exhibits no molecular symmetry, the symmetric forms being either transition states or higher-energy forms. It is a long-standing fallacy among

both experimental and theoretical researchers that if a molecule *may* assume a highly symmetric form, then it *must* do so. We anticipate that our results will help to overcome this misconception.

This work is described in detail in a forthcoming publication in the Journal of the American Chemical Society (scheduled for publication in July, 1986).

D. O-Methyl Methylphosphonofluoridate

This compound, $(\text{CH}_3)_2\text{P}=\text{O}(\text{F})(\text{OCH}_3)$, is the simplest example of a nerve agent. It is the methyl ester of O-methyl methylphosphonofluoridic acid. Although it is a relatively weak anticholinesterase agent itself, the higher esters include several important enzyme inhibitors such as the isopropyl ester (sarin) and the pinacolyl ester (soman). An electron-diffraction study of its molecular structure has been reported¹⁰, so that it also provides another test of our computational methods.

We have determined the complete molecular structure, including the equilibrium conformation in STO-3G* and 3-21G* basis sets. Our finding may be summarized as follows:

1. The bond lengths and angles are again quite similar to those found for the phosphorus acids and esters described above, and in reasonable agreement with the electron diffraction results (except for a few gross errors in the bond angles about the phosphorus atom in the latter, likely due to errors in manipulating the experimental data).
2. Our computed structure places the methoxy methyl group adjacent to the phosphoryl oxygen, as in methyl phosphinate. It is however slightly rotated toward the fluorine, as might be expected on electrostatic grounds given the greater negative charge generally found on fluorine compared to hydrogen. The electron-diffraction study reported the methyl group to be opposed to the phosphoryl oxygen. We however find no minimum in the energy corresponding to such a structure. After careful study of the energy surface in both basis sets we concluded that the reported experimental results are in error on this crucial point.

3. Expanding the torsional energy in a Fourier series again resulted in a dominant V_1 term.

4. The minimal-energy structure again exhibits the minimal molecular dipole moment. So this represents yet another species whose equilibrium structure is stabilized by dipolar electrostatic interactions.

We have published a description of this work in *The Journal of Molecular Structure (Theochem)* 122, 179 (1985).

E. Esters Directly Related to Chemical Defense

Employing the bond lengths and bond angles (except for torsional angles) we computed the energies of selected conformations of four compounds which are active acetylcholinesterase inhibitors: the ethyl, isopropyl and pinacolyl esters of O-methyl methylphosphonofluoridic acid (the latter two generally referred to as sarin and soman respectively) and O-ethyl N-dimethylphosphoramidocyanidate (tabun). The results may be briefly summarized as follows:

1. The O-isopropyl ester prefers a structure in which the α hydrogen of the isopropyl group is adjacent to the phosphoryl oxygen.

2. In the case of the two O-ethyl esters, there are two α hydrogens. The two stable conformations both correspond to a hydrogen adjacent to the phosphoryl oxygen. These are essentially equal in energy.

3. Soman, the pinacolyl ester, also displays a structure in which the α hydrogen is adjacent to the phosphoryl oxygen.

4. The energy barrier for rotation of the ester side chain about the P-O bond, which tends to hold the molecule in its equilibrium conformation, is appreciable in each case, generally in the range of 8 to 10 kcal/mol.

We conclude that each of these species are constrained to an equilibrium structure that is likely to be due to the same types of forces as seen in the smaller phosphorus acids and esters we have studied, and held in this form by forces that would probably prevail under physiological conditions as well

as in the gas phase. Hence there is a good possibility that these same conformational forces have a role in determining the activity of anticholinesterase agents *in vivo*.

Most of this work has also been described in *Proceedings of the 1983 Scientific Conference on Chemical Defense Research*, (US Army Chemical Research and Development Center, Aberdeen Proving Ground, Maryland, 1984, 159).

F. Compounds With P-O-P Linkages

During the last few months of the research project, and as a logical extension of our systematic studies of phosphorus oxyacids and their esters, we have studied the molecular structures of some simple compounds exhibiting a P-O-P linkage. This linkage plays a role of supreme importance in the structural chemistry of phosphorus compounds, somewhat like the role played by the C-C bond in the chemistry of carbon compounds. It also plays the key role in the transport and storage of energy in living cells. (In addition pyrophosphates containing this linkage such as tetraethylpyrophosphate, TEPP, are very active acetylcholinesterase inhibitors.)

We have approached this problem in the same way as was employed for our studies of the simpler phosphorus acids and esters: we first examined one of the simplest compounds of this type, μ -oxo-diphosphine, $\text{H}_2\text{P}-\text{O}-\text{PH}_2$, in a number of basis sets including the 66-311G** basis for comparison with the smaller sets. We then studied the larger compounds diphosphinic acid (or phosphinic acid anhydride, $\text{H}_2(\text{O}=\text{P})-\text{O}-(\text{P}=\text{O})\text{H}_2$), diphosphonic acid (or pyrophosphonic acid, $\text{H}(\text{OH})(\text{O}=\text{P})-\text{O}-(\text{P}=\text{O})(\text{OH})\text{H}$), and diphosphoric acid (or pyrophosphoric acid, $(\text{OH})_2(\text{O}=\text{P})-\text{O}-(\text{P}=\text{O})(\text{OH})_2$). In each case we carried out detailed structure optimizations at a number of points on the energy surface in order to identify all stable conformations, thus providing a rigorous basis for subsequent computations of the structure or energy. Finally, from our earlier computed energies of phosphinic, phosphonic and phosphoric acids we computed the enthalpies of the dimerization (dehydration) reactions to form the P-O-P linkage in each species.

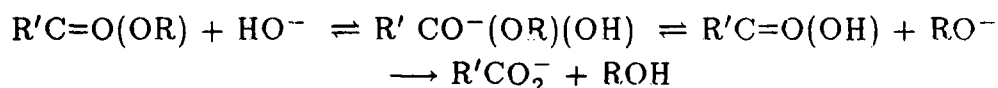
This work is currently being completed and prepared for publication.

IV. Structures and Energetics Relevant to Ester Hydrolysis

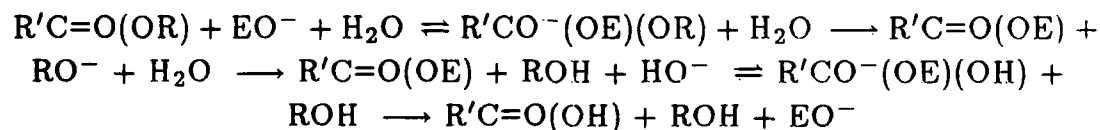
A. Orthoformates as Model Hydrolysis Intermediates

The key steps in the hydrolysis of esters (and peptides) either by enzymes or in aqueous solution involve formation of a tetrahedral intermediate species¹. The intermediate species then breaks down to form the final products: an alcohol and either an acid or its conjugate base. Inhibitors, which block the enzyme active sites that are essential for hydrolysis, mimic the molecular structure of the intermediate, thus tricking the enzyme into binding the inhibitor tightly to its active site. The inhibitor however breaks down relatively slowly, thus disrupting the function of the enzyme by blocking the binding site. Therefore an alternative way to describe the structural requirements of an effective inhibitor is to study the molecular structures of the tetrahedral hydrolysis-intermediate species that bind to the same site.

We performed a detailed study of the molecular structures and energetics of the simplest prototype molecules occurring in the base-catalyzed or enzymatic hydrolysis of esters. For base catalysis in solution, it is generally thought that the pertinent steps are



and, for the enzymatic hydrolysis



where R' and R are organic groups and EO⁻ stands for a deprotonated serine residue of the enzyme. The three intermediate model compounds, R'CO⁻(OR)(OR''), on which this study is focused correspond to R' = H in each of three cases: R = R'' = H for the simplest one, which was studied the most thoroughly; R = CH₃ with R'' = H for another; and R = R'' =

CH₃ for the last one. They may be considered formally as adducts of HO⁻ with HC=O(OH), HO⁻ with HC=O(OCH₃) or equivalently CH₃O⁻ with HC=O(OH), and CH₃O⁻ with HC=O(OCH₃).

Our conclusions based on this study may be summarized as follows:

1. The three compounds we studied, [HCO(OH)₂]⁻, [HCO(OH)(OCH₃)]⁻ and [HCO(OCH₃)₂]⁻, were found to be surprisingly stable species — both in the sense of having no dissociative normal modes and also in the sense of having enthalpies and free energies appreciably lower than the other species in the overall reaction. For example [HCO(OH)₂]⁻ is much lower in enthalpy and free energy than HCO(OH) + HO⁻. The widespread belief¹¹ that these are ephemeral high-energy species or transition states is clearly erroneous.

2. The conformations of all three species have are characterized by the hydroxy hydrogen or methoxy methyl group being adjacent to the C-O⁻.

3. The Fourier expansion of the torsional potential shows a quite different type of potential than that seen for the phosphorus acids and esters. This type of potential is characterized by a ratio of the first two Fourier coefficients $V_1:V_2 = 2:1$, both for the hydrogens and methyl groups. This potential, which is strongly attractive for torsional angles near zero but quite small elsewhere, we have termed a "monopolar" potential, since it has the behavior of the attraction between two point charges. Such behavior is the opposite of that expected from stereoelectronic effects, which had been widely postulated¹² as determining the structures of these species based on indirect deductions from experimental kinetic data.

4. The barrier to internal rotation is quite high in all these species — on the order of 10 kcal/mol — which together with the Fourier coefficients points to internal hydrogen bonding (plus an analogous interaction for the methyl groups).

5. The molecular structures of all three of the prototype hydrolysis intermediates we studied exhibit remarkable similarities to those we computed for organophosphorus inhibitors. Not only is the central atom nearly tetrahedrally coordinated in both cases, but also bond lengths to this atom (carbon

in the former case and phosphorus in the latter) become roughly comparable. In other words, the anionic nature of the intermediate, containing a second-period element, causes it to have bond lengths similar to the neutral inhibitor compound containing a third-period element. The structural similarities even extend to details of the molecular conformation, such as the torsional angles of an adjacent methyl group. We believe this proves our hypothesis that studying the molecular structures of prototype hydrolysis intermediate species is a practicable way to infer the structures of the inhibitor compounds that bind in their place to enzyme active sites.

6. With respect to the technical details of the computations, we demonstrated that relatively small 4-31G* basis sets give quite accurate results for the structures for species closely related chemically to the intermediate species themselves. But with respect to relative energies of anionic species basis sets of this type give very poor results unless augmented by carefully chosen sets of diffuse functions. This observation, which had been made previously with regard to protonation of anions, we showed to be the case in general. When the proper set of diffuse functions is added, an acceptable accuracy may be readily obtained. In computing enthalpy differences in reactions, such as the addition reactions to form intermediates or the overall hydrolysis reactions, corrections due to zero-point and thermal motions were included. However it was found that electron-correlation corrections to the energy at the MP2 level (second-order Møller-Plesset perturbation approximation) did not consistently give better agreement with experiment for reactions involving neutral species, and it almost invariably gave significantly lower accuracy for anionic reactions.

In summary, we have shown that detailed *ab initio* study of the molecular structures and energies of prototype intermediate species sheds considerable light on the requirements for efficient binding to the enzyme, both by the enzyme's natural substrates and its inhibitors. We have also shown that, with the possible exception of the Møller-Plesset expansion, computational methods we have employed are of sufficient accuracy and reliability for such studies.

We have described this work in detail in a forthcoming paper in the *Journal of the American Chemical Society* (scheduled for publication in July,

1986). Structural similarities among both these intermediate species and also organophosphoryl compounds are reviewed in *Proceedings of the 1985 Scientific Conference on Chemical Defense Research*, (US Army Chemical Research and Development Center, Aberdeen Proving Ground, Maryland, 1986, 521).

B. Quantitative Protonation Enthalpies of Anions

The above results raised a number of questions about the accuracy of *ab initio* computations involving anions. We therefore carried a systematic study of protonation enthalpies, employing a number of different basis sets augmented by diffuse functions, and with the electron correlation obtained with several different types of approximations. The specific anions were HO^- , CN^- , CH_3^- , CH_3O^- , HCOO^- , and NO_3^- . These may be compared directly with experimental enthalpies, which have been accurately measured and tabulated¹³. Our conclusions resulting from this study may be summarized as follows:

1. For highly accurate computation of protonation enthalpies, correct treatment of the vibrational corrections (as well as rotational, translational and *PV* terms) is essential. We found that in obtaining accurate values for these corrections, the size of the basis set is not as important as the inclusion of electron correlation. For example for the protonation of HO^- , *i.e.* for the reaction $\text{HO}^- + \text{H}^+ \rightarrow \text{H}_2\text{O}$, the 4-31G basis at the SCF level gives a total correction of 9.5 kcal/mol., the 4-31G* basis gives 9.4, but the MP2 value in the 4-31G* basis is 8.7.

2. As expected from our studies on species involved in ester hydrolysis, the MP2 approximation gives quite inaccurate results, being almost invariably worse than either higher-level approximations or even the SCF approximation. MP3 is satisfactory and the fourth-order correction, MP4 (with single, double and quadruple excitations) and the conventional configuration-interaction procedure (with double excitations) both give results in excellent agreement with experiment if the proper basis set is employed.

3. Basis sets at least as large as 6-311G** (three independent s and p valence functions, p functions on hydrogen atoms and a set of d functions added to other atoms) are required to obtain a suitable result, particularly for an accurate correlation-energy correction.

4. Corrections we have termed "indirect" due to the effect of diffuse orbitals or electron correlation on the optimized molecular structure were found to be relatively small - typically about 0.1 kcal/mol.

The results we obtained were considerably more accurate than any those of any previous systematic study. For example for the protonation of HO^- we compute at the MP4 level an enthalpy at 25° C. of -392.2 kcal/mol, which agrees well with the experimental value¹³ of -390.8 ± 1 .

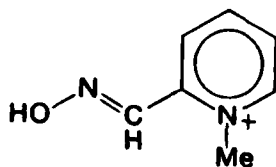
Our publication describing this work is currently in press in the Journal of Physical Chemistry.

V. Structural Properties of 2-PAM

The third and final type of compound studied as a part of this project which are related to the functioning of esterase enzymes are the reactivating antidotes. Antidotes of this type are thought to regenerate the enzyme active site by first binding electrostatically to a point adjacent to that site, then hydrolyzing the inhibitor that is bound to the enzyme. The hydrolysis products then release the enzyme active site, hence reactivating it.

As might be expected from the assertion that the antidote occupies a different binding site (presumably with a much different enzyme structure) from the site that binds the inhibitor, antidote compounds exhibit very different molecular structures from the enzyme inhibitors, substrates or intermediates as discussed in the two preceding sections. The most widely employed reactivating antidote for acetylcholinesterase inhibitors is pralidoxime (pyridinium,

2-[(hydroxyimino)methyl]-1-methyl halide salts, or 2-PAM. Its structure is generally written as



Most of the other known antidotes¹⁴ similarly contain cationic methylpyridine groups with oxime side chains in the 2 or 4 position, and are therefore widely believed to have essentially the same mode of action as does 2-PAM. The mechanism is thought to entail electrostatic binding to a negatively charged site in the enzyme via the overall positive charge on the 2-PAM cation and deprotonation of the oxime moiety, which then reacts with the phosphorus atom of the inhibitor.

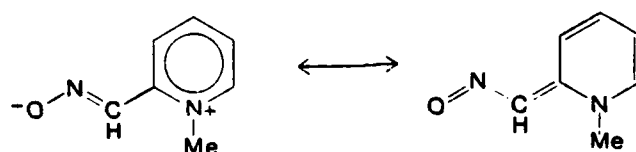
There are clearly two molecular properties of PAM relevant to its efficacy as an antidote that are amenable to accurate characterization by *ab initio* methods: the molecular structure, particularly the conformation of the reactive side chain, and the location of the positive charge that is required for binding to the enzyme. There is an additional consideration in that the deprotonated form is the actual reactivating agent, but it is not currently known whether this conjugate base species is formed before or after the 2-PAM binds to the enzyme. Therefore it is essential to compute the molecular structure, conformations and charge distribution of the base form as well.

The complete molecular structures of both 2-PAM and its conjugate base were optimized in two basis sets: STO-3G and 3-21G. Employing these two sets simultaneously provides a test of the sensitivity of the computed results to the choice of basis set, and gives a measure of the suitability of the smaller one for obtaining accurate results on even larger chemically related species. Our results may be briefly summarized as follows:

1. The equilibrium conformation of 2-PAM is the completely E or *trans* form shown above. In each case it is of C_s symmetry, the plane of the pyridine ring being a symmetry plane. It had earlier been postulated¹⁵ that in order to have its observed activity in comparison to chemically related compounds

such as 3-PAM it must have the *Z* or *cis* orientation about the C=N bond. However it long been known from x-ray diffraction studies¹⁶ to be in the *E* conformation in crystals. Since we have now shown that it is in the *E* in isolated molecules as well, it is very likely to be in the same form in solution and *in vivo*.

2. Optimization of the conjugate base of 2-PAM, formed by removing the proton from the OH of the oxime side chain, showed that it has a much different type of structure than does the protonated species. It may be described as a superposition of the two resonance structures



with the form on the right predominating. It thus exhibits a relatively homogeneous charge distribution, rather than being a zwitterion. However it again is of C_s symmetry and has an *E* minimum-energy conformation, as does the protonated form.

3. We compute 2-PAM to possess only one stable conformer, explaining why attempts to synthesize the *Z* form chemically have been unsuccessful. The conjugate base has at most only a shallow energy minimum in the *Z* form which, as with the protonated form, will likely prove impossible to isolate synthetically.

4. A Fourier analysis of the energy as a function of the torsional angles of the side chain for 2-PAM and its conjugate base shows that both V_1 and V_2 (reflecting in this case steric repulsion with the methyl group and π bonding respectively) stabilize the planar *E* form. V_1 predominates in each case, accounting for the relative stability of the *E* over the *Z* arrangement. We conclude that the preferred orientation of both 2-PAM and its conjugate base and their overall molecular rigidities, which in turn are important to physiological activity, are due in roughly equal measure to steric repulsion by the neighboring methyl group and to the delocalized π bonding possible only in this type of conjugated system.

5. The charge distributions of both species, as assayed by the gross Mulliken populations computed in the STO-3G basis, show that the quaternary nitrogen is negatively charged, contrary to the implication of the conventional structural formulas, as above. The positive charge is approximately equally distributed among the hydrogen atoms, both of the methyl group, side chain and pyridine ring. Therefore biochemical mechanisms that postulate the quaternary pyridine nitrogen as being the precise site of electrostatic binding to an enzyme, by virtue of a localized positive charge, are clearly in error.

6. As a check on the techniques employed to compute molecular structure, the computed bond lengths and angles may be compared with the most recent x-ray diffraction results.¹⁶ In the 3-21G basis the average error in bond lengths is only 0.014Å, and in bond angles 0.82°. However the simple STO-3G basis is nearly as accurate, with an average error of 0.022Å in bond lengths and 0.61° in bond angles.

It is a surprising result, although one we have observed often during this research project, that small basis sets give increasingly more accurate structural parameters as the size of the molecule under study increases. In other words very large species, when fully geometrically optimized but employing a small STO-3G basis, have smaller average errors than do smaller molecules with similar electronic structures in the same basis. Although the theoretical origins of this are unclear it may result from basis set superposition effects, *i.e.* basis functions that are not sufficient to characterize accurately one region of the molecule are assisted by the tails of basis functions centered on remote parts of the molecule. In any event this observation bodes well for the future of accurate *ab initio* computations on such relatively large species.

VI. Other Related Studies

In this section we summarize the results of three other studies which are only indirectly related to those described above. The common feature

is the ongoing need to test existing techniques for *ab initio* computations on structures and properties of small molecules and molecular ions.

A. *Ab Initio* Intensities of Infrared Bands

Recent advances in the computer programs available for researchers in quantum chemistry now permit the rapid and accurate computation of all the vibrational frequencies in molecules of moderate size. This is proving to be a valuable technique for assigning and interpreting vibrational spectra, particularly those associated with unstable species such as are often studied by means of matrix-isolation techniques. The principal theoretical development has been computer code that evaluates analytically both the first and second derivatives of the energy with respect to molecular deformations. This code is contained in the GAUSSIAN 82 package and its subsequent versions.

Of course to interpret the spectrum the relative intensities of the transitions are nearly as important as the frequencies. Although there has recently been some progress in programming analytic expressions for the infrared intensities at the SCF level, correct and reliable versions of these programs are not widely available as yet. Therefore we have been developing, in collaboration with Profs. L. J. Schaad and B. A. Hess, Jr. of this department, a simple procedure to compute infrared and raman intensities at the SCF and MP2 levels of approximation. This is done by embedding the molecule in a static electric field formed by sets of point charges (which are fixed at a considerable distance from the molecule so that no bonding or charge transfer can occur). Analytic computer code such as contained in GAUSSIAN 82 may then be employed to compute the forces on the nuclei with and without this applied field.

Infrared intensities are given, to a good approximation, by the derivatives of the x, y, and z components of the dipole moment μ with respect to the normal vibrational modes. For mode j ,

$$\left| \frac{\partial \mu}{\partial Q_j} \right|^2 \equiv \left(\frac{\partial \mu_x}{\partial Q_j} \right)^2 + \left(\frac{\partial \mu_y}{\partial Q_j} \right)^2 + \left(\frac{\partial \mu_z}{\partial Q_j} \right)^2$$

where μ_x , μ_y and μ_z are the three components of the dipole moment vector,

and Q_j is the normal coordinate of the vibration. However since the dipole is just the derivative of the energy with respect to an electric field,

$$\frac{\partial \mu_a}{\partial X_i} = - \frac{\partial}{\partial F_a} \left(\frac{\partial E}{\partial X_i} \right)$$

where X_i is any nuclear displacement, F_a is the x, y or z component of the applied electric field, and E is the total molecular energy. Here $\partial E / \partial X_i$ is simply the i^{th} component of the gradient in cartesian space. Hence computing the forces as a function of applied field gives the information needed to compute the infrared intensities. The important point is that this need be done only three times, one for each of the three field directions, regardless of the number of vibrational modes. Similar expressions apply to raman intensities, which depend on the second derivative of the forces with respect to the field.

As an initial test case we computed the infrared intensities of ethylene in a variety of basis sets, and employing several field strengths and positionings of the point charges. Although the results were found to depend markedly on the basis set, they were remarkably independent of the strength and other details of the way in which the field is created.

In Figure 2 below we show in the bottom section the *ab initio* infrared spectrum of ethylene computed in the STO-3G basis, and compare the intensities computed by means of the

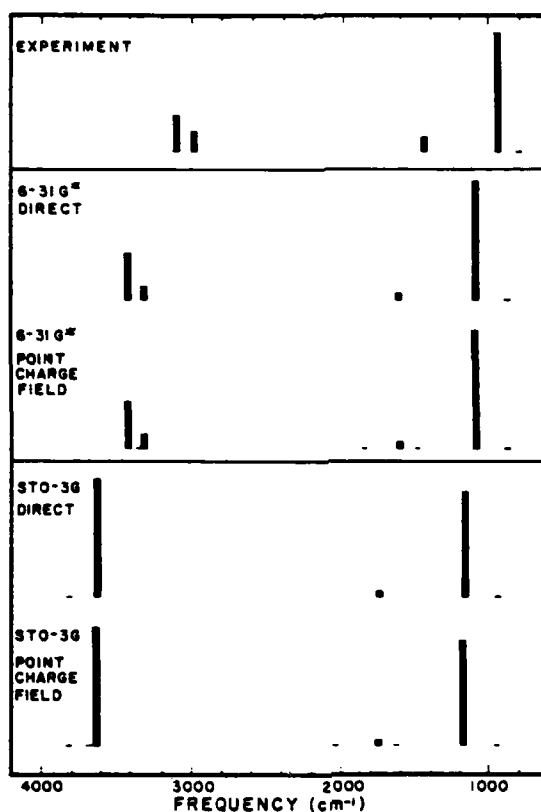


Figure 2

applied field (below) with those laboriously computed by evaluating the dipole moment expectation value at a large number of deformed geometries (above). In the middle section the same comparison is presented for the much larger 6-31G* basis. This may be seen to be in excellent agreement with the experimental relative intensities, in the top section. Most of the error in the frequencies may be shown to be due to the correlation energy. We have found that inclusion of electron correlation at the MP2 level (see section IV.B. above) removes most of the remaining error in computed vibrational frequencies. We are currently performing a similar study of raman spectra.

Our first paper on this topic, describing the procedure more fully, has recently been published in *The Journal of Chemical Physics* 83, 5348 (1985).

B. Computed Structures of $C_4H_4^{2+}$ and $C_4H_4^{2-}$

These two species, whose formulas are usually written as



are of considerable interest in the theory of molecular electronic structure since they are conjugated hydrocarbons whose valence electrons satisfy the Hückel $4n+2$ rule but, based on semiempirical arguments and experimental studies of their derivatives, they are nevertheless considered not to be aromatic¹⁷.

This study was undertaken in collaboration with Profs. Schaad and Hess of this department. We optimized the molecular structures of both species at the SCF level in both a 3-21G and a 6-31G* basis. The dianion, being an allylic species and hence with possibly a large electron-correlation contribution to its structure, was optimized in the larger basis at the MP2 level of approximation. Both species were studied both with and without counterions: an F^- for the dication and a Li^+ for the dianion. The principal results may be summarized as follows:

1. Both species were found to be nonplanar and hence nonaromatic. The optimized structures of the dication (on the left), dianion (on the right), and for comparison the neutral cyclobutadiene (in the middle) are shown below.

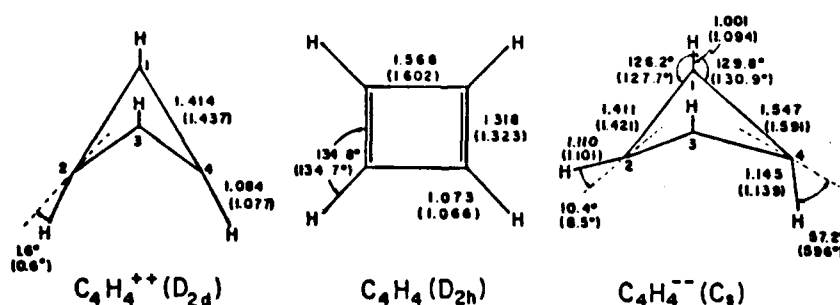
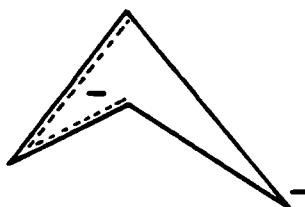


Figure 3

The values in parentheses refer to those computed in the 3-21G basis, the others to the 6-31G* basis.

2. Note that the 3-21G and 6-31G* basis sets give very similar results, as did those with and without a counterion. The primary effect of the counterion is only a slight bending away by the hydrogen atoms. Although of course this was not unanticipated, these are examples of the sort of technical details that must always be checked out in order to obtain reliable results in a study of this type.

3. The dianion was found to have the unusual structure characterized by a localized negative charge



the difference between the two types of C-C bond lengths being 0.14Å in the SCF case.

4. A remarkably large change is seen in the structure of the dianion when optimizing at the MP2 level, the difference in the C-C bond lengths decreasing to 0.05Å.

5. Finally we note that these structures, particularly that of the dianion, are of remarkably low symmetry. This is another example of the point discussed above in section III.C. and which is recurrent theme in the results of this project, that a molecular connectivity that *permits* a high symmetry (D_{4h} in this case) does not imply that the molecule *must* exhibit that symmetry.

We have recently published an account of this research in *The Journal of Organic Chemistry*, 50, 5859 (1985).

C. Computed Structures of Tartaric Acid

The final study of this series was undertaken in collaboration with Prof. P. L. Polavarapu of this department. It is indirectly related to our work, described in section IV, on the equilibrium structures of acids and esters. We noted above that it now seems to be invariably the case that oxyacids, whether of phosphorus or carbon, assume a structure in which the acidic hydrogen is adjacent to a carbonyl or phosphoryl group. Tartaric acid, $(\text{HOOC})(\text{CHOH})(\text{CHOH})(\text{COOH})$, is a useful test of our concepts of the ways in which such compounds will be configured, due to the large number of torsional angles (seven) and hence the large number of possible stable forms. This data had not previously been determined either theoretically or experimentally, although Prof. Polavarapu has recently obtained vibrational circular dichroism measurements which provide indirect information.

We optimized the molecular structure of tartaric acid in the STO-3G basis, with no constraints except that the molecule have the experimental C_2 symmetry. This was repeated at a large number of initial conformations. The results may be summarized as follows:

1. Each stable structure exhibited each hydrogen atom adjacent to an oxygen. Not surprisingly each carboxyl group always has a planar *cis* geometry.
2. The structures with the COOH groups *trans* about the central C-C bond are always lower in energy than other arrangements.
3. Of the *trans* COOH forms there are four possibilities: the central two COH groups can be oriented either toward the nearest or next-nearest carboxyl group to form a five- or six-membered ring respectively, and for each of these it may be nearest the C=O or the C-OH of the carboxyl. These types of structures, as well as their computed relative energies, are shown in Figure 4 below.
4. Note that the lowest-energy forms are those in which the hydrogen is adjacent to the C=O, precisely analogous to the phosphorus oxyacids described above in section III.B., in which the hydrogens are always adjacent to a P=O group. Hence it seems likely that the theoretical picture that explains the phosphorus acids applies to this case as well. Such consistently

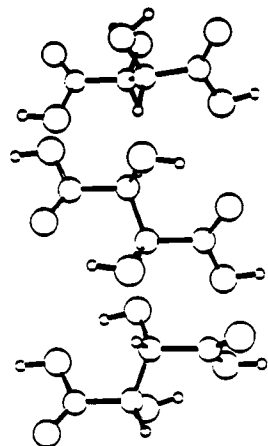
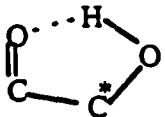
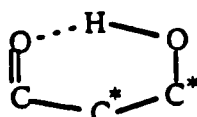
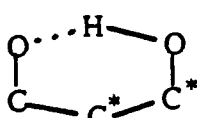
	Type of Intramolecular Hydrogen Bonding	Relative Energy (kcal/mol)
		0
		0.5
		0.6

Figure 4

recurring structural trends among chemically dissimilar species is one of the most interesting qualitative results of this research project.

5. Finally, the conclusion that tartaric acid has three stable low-energy structures of comparable energy is in precise agreement with the interpretation of the experimental spectra.

This work, including both theoretical and experimental results, has recently been submitted for publication.

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VIII. Publications Resulting from This Grant

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6. C. S. Ewig, *Proceedings of the 1985 Scientific Conference on Chemical Defense Research*, (US Army Chemical Research and Development Center, Aberdeen Proving Ground, Maryland, 1986, 521-526. Comparison of Some Computed Structures of Compounds that Interact With AChE.

7. J. R. Van Wazer and Carl S. Ewig, *J. Am. Chem. Soc.* (in press). Ab Initio Structures of Phosphorus Acids and Esters. 2. Methyl Phosphinate, Dimethyl Phosphonate and Trimethyl Phosphate.

8. C. S. Ewig and J. R. Van Wazer, *J. Am Chem. Soc.* (in press). Ab Initio Studies of Molecular Structures and Energetics. 1. Base-Catalyzed Hydrolysis of Simple Formates and Structurally Related Reactions.

9. C. S. Ewig and J. R. Van Wazer, *J. Phys. Chem.* (in press). Ab Initio Reaction Enthalpies for Some Gas-Phase Acid-Base Neutralizations.

10. T. Chandramouly, C. S. Ewig and P. L. Polavarapu (submitted for publication). Vibrational Circular Dichroism and Conformations of Tartaric Acid and its Esters.

11. C. S. Ewig and J. R. Van Wazer (submitted for publication). An Ab Initio Study of the Structure and Bonding of Pralidoxime and its Conjugate Base.

12. J. R. Van Wazer and C. S. Ewig (in preparation) Ab Initio Structures of Phosphorus Acids and Esters. 3. Diphosphine-mu-oxide and Diphosphinic, Diphosphonic and Diphosphoric Acids.

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